REMARKS / ARGUMENTS

By the present amendment, claim 1 has been amended as described below. The amendments to the claims have been made without prejudice and without acquiescing to any of the Examiner's rejections. Applicants reserve the right to pursue any of the deleted subject matter in a further divisional, continuation or continuation-in-part application. No new matter has been entered by the present amendment and its entry is respectfully requested.

The office action dated February 19, 2010 has been carefully considered. It is believed that the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

35 USC 103(a)

The Examiner rejected claims 1, 32 and 39-40 under 35 USC 103(a) as being unpatentable over US Patent Publication No. 2004/0142488 to Gierde et al. ("Gierde") in view of US Patent Publication No. 7,379,820 to Sukits et al. ("Sukits"). The Examiner alleges that Gierde teaches steps (a) to (c) of present claim 1 at paragraphs [137] and [195-201] and that Sukits describes a series of protein pairs that associate together *in vivo* through electrostatic interaction that can be separated by using NaCl to disrupt the electrostatic interaction at Column 18, lines 30-38. Accordingly, the Examiner is of the opinion that one of ordinary skill in the art would have found it obvious to modify Gierde's method to investigate protein-protein interactions in a multi-protein complex using the affinity chromatography assay and that by combining Gierde and Sukits, Gierde's method would be modified to include a NaCl elution step on an immobilized protein-protein complex. Applicants respectfully disagree for the reasons that follow.

Applicants respectfully submit that for the presently claimed methods, not only is the bond between the first ligand and the second ligand a protein-protein bond, but the bond between the second ligand and the affinity matrix is also a protein-protein bond. Therefore, at step 1(d), one of ordinary skill in the art would expect that increasing the ionic strength would lead not only to dissociation of the bond between the second ligand and the first ligand, but to dissociation of the bond between the affinity matrix and the second ligand. The Applicants were the first to propose and demonstrate that an intact protein complex can be immobilized noncovalently on an affinity

matrix in such a way that would allow for the selective separation of the proteins (i.e. first ligands) that interact with the immobilized affinity tagged protein (i.e. second ligand) while the latter remains immobilized. For clarity, Applicants have amended claim 1 to recite that the immobilization in step 1b) is non-covalent. Further, the Applicants were first to propose and demonstrate the usefulness and desirability of such separation, i.e. the elimination of the dynamic range problem [0009-0015 and 0077- FIG. 21] in the present application.

In contrast to the presently claimed method, both Gierde [0083-0092] and Sukits [column 18, lines 30-38] teach to first purify the second ligand to homogeneity and then couple it to a solid support by covalent bonds, before applying biological sample containing the first ligand. Therefore, Gierde and Sukits teach away from the presently claimed method in two ways:

(1) The Examiner points to column 18, lines 30-38 of Sukits: "Similar to many of the proteins in the death domain superfamily, RIP DD contains a charged surface (FIGS. 3A and 3B) where titration with NaCl disrupted the interaction between RIP DD and TRADD indicating that the homotypic interaction is at least in part electrostatic (FIG. 4A). The electrostatic interaction between RIP DD and TRADD is consistent with the proposed interactions of FAS DD with FADD DD (Jeong et al., 1999) and of TNFR-1 DD with TRADD DD (Telliez et al., 2000)." However, Applicant respectfully submits that Sukits is referring to classic affinity chromatography. In fact, Applicant respectfully submits that in all three studies (Sukits, Jeong and Telliez) a classic affinity chromatography is used. *I.e.* the second ligand is overexpressed in a heterogeneous organism and, after its purification, is covalently coupled to solid support to form an affinity matrix. After applying solution containing the first ligand, the salt concentration is increased in order to determine whether the increase of the ionic strength would lead to disruption of the electrostatic bonds and cause the dissociation of the protein-protein complex.

Thus, both Gierde [0083-0092] and Sukits [column 18, lines 30-38] describe classic affinity purification of proteins and, therefore, Sukits [column 18, lines 30-38] does not remedy the deficiencies of Gierde [0083-0092]. Step 1(b) in the presently claimed method states: "Non-covalently immobilizing the second ligand on an affinity matrix" which is in no way equivalent to "covalently binding the second ligand on an affinity matrix". When a protein is covalently bound to solid support it becomes part of the affinity matrix (although the most important one) and cannot be regarded as "second ligand" any more.

(2) Both Gierde [0083-0092 and 0137] and Sukits [column 18, lines 30-38] teach the formation *de novo* (*in vitro*) of an artificial complex between the first ligand and second ligand and sequential disruption of this artificially formed complex. The present inventors are the first to demonstrate directly that *in vivo* formed transient complexes are associated by predominantly electrostatic bonds and that the disruption of the electrostatic bonds is enough for the disintegration of the complex. Complexes that are studied by Sukits, Jeong and Telliez (RIP DD-TRADD, FAS DD -FADD DD and TNFR-1 DD with TRADD DD) are artificial complexes formed *de novo* (*in vitro*). The immobilized proteins (second ligand) are not present in full length and are not synthesized in their respective endogenous organisms. A person skilled in the art would know that *in vivo* two proteins would associate in a different way than *in vitro*. The immobilized proteins (second ligand) are not present in full length and are not synthesized in their respective endogenous organisms. See the attached paper of Jeong et al., 1999, page 16338, bottom left – "Mutations and Measurements of Binding Affinity". A person skilled in the art would know that even though the truncated forms of two proteins associate electrostatically *in vitro* and increasing the ionic strength dissociates the complex, it does not follow automatically that the same two proteins associate in the same way *in vivo* and that they can be separated upon salt elution.

In addition, Applicants respectfully submit that two ligands can be associated by electrostatic bonds but it does not necessarily mean that they can be separated from one another by increasing the ionic strength of the medium for at least two reasons:

- the electrostatic bonds can be inaccessible to the small ions (resulting from the increased ionic strength);

- beside the electrostatic bonds, the two ligands can be associated by other bonds, i.e. hydrophobic bonds, and even if the electrostatic bonds are disrupted, the two ligands can remain bound to each other.

Yet, in both cases the association between the two ligands can be most appropriately described as "associated by electrostatic bonds" because the energy of the electrostatic bonds, especially the Coulomb forces, is much bigger than the energy of the non-electrostatic bonds. As an example, in "Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution." by Cramer P, Bushnell D, Kornberg R. (Science, 2001, http://www.sciencemag.org/cgi/reprint/292/5523/1863.pdf, attached), Table 2 - "Subunit interactions", Rpb1 forms 6 salt bonds, i.e. electrostatic bonds, with Rpb2, 5 salt bonds with

Rpb5, and three salt bonds with Rpb6 and Rpb8. In total, there are 45 electrostatic bonds between the 12 subunits. However, after immobilizing the RNAP II complexes on affinity matrix by affinity tagged Rpb1, the other 11 subunits of the core RNA Polymerase II complex are present at approximately the same molar amount as Rpb1. This amount is much higher (with at least an order of magnitude) than the amount of the transcription factors. Yet, the 11 subunits of the core RNA Polymerase II complex (other than the immobilized Rpb1), are not eluted upon increasing the ionic strength (see the results in Figures 3, 4, 6, 7, 8, 10, 11, 12). If one of ordinary skill in the art follows the teachings of Rigaut and Gierde, he would expect Rpb2, Rpb5, Rpb6 and Rpb8 to be eluted/ desorbed upon increasing the ionic strength.

Further, in step 1(c) of the presently claimed method, prior to salt elution, the only *de novo* formed protein-protein interaction is the one between the affinity matrix and the second ligand. Therefore, the prior art of Gierde, Sukits, Jeong and Telliez teaches one of ordinary skill in the art that upon increasing the salt concentration the most obvious result would be the separation of the second ligand from the affinity matrix (with or without the separation of the second ligand from the first ligand), which clearly teaches away from the presently claimed method.

The Examiner also cites the disclosure at paragraphs [195-201] of Gierde as disclosing that the biomolecule is a multi-protein complex and that one or more constituents of the multi-protein complex can be recovered. Applicant respectfully submits that this section of Gierde is not citable against the present application. Gierde was published on July 22, 2004 and filed on January 8, 2004. The present application has an international filing date of July 30, 2004 and claims priority dating back to US Provisional 60/494,811 filed on August 14, 2003, which predates the Gierde application. Gierde also claims priority to other previously filed applications, US 10/620,155 filed July 14, 2003, US 60/465,606 filed on April 25, 2003 and US 60/396,595 filed July 15, 2002. Applicant has reviewed these three Gierde priority documents and respectfully submits that these three documents do not provide support for paragraphs [195-202] of the presently cited Gierde application. Thus, Applicant respectfully submits that paragraphs [195-201] were filed and published after the present application's first priority document, US 60/494,811 and are not citable against the present application.

Even if Gierde [195-201] were citable, which we disagree with, Gierde [0137 and 0195-0201] and Sukits [column 18, lines 30-38] do not teach or suggest that the second ligand (the protein that is immobilized on the affinity matrix) must remain immobilized on the affinity column during

the elution of the first ligand. As described in the present application, desorption of the second ligand must be avoided. Otherwise, a severe dynamic range problem will be encountered, which will make the identification of the first ligand impossible. The mere statement by Gierde at paragraph [0201] that "some subset of the complex is released while the rest remains adsorbed", is very vague and does not provide any guidance that would lead to the presently claimed method. In this regard, neither Gierde [0137 and 0195-0201] nor Sukits [column 18, lines 30-38] teach or suggest that the elution agent should be chosen in such a way that it favours the separation of the first and second ligand, while not affecting the bond between the second ligand and the affinity matrix. In fact, Gierde provides two examples that teach away: "For example, by decreasing the polarity of a desorption solvent hydrophobic interactions will be weakened-inclusion of reducing agent (such as mercaptoethanol or dithiothrietol) will disrupt disulfide bridges."

A - Decreasing the polarity of a desorption agent would weaken not only the hydrophobic interactions within the protein complex but the bond between the affinity matrix and the immobilized second ligand, as well. (Hydrophobic forces are present in every protein-protein association). Thus separation of the first ligand from the second ligand as described in the present application would not be accomplished.

B - Inclusion of reducing agent would weaken indiscriminately the disulfide bridges and would affect the permanent protein-protein association to a much bigger extent than the transient (predominantly electrostatic) interactions. Thus the dynamic range problem would not be avoided.

In view of the above, Applicants respectfully submit that claims 1, 32 and 39-40 are inventive over Gierde in view of Sukits.

The Examiner rejected claim 37 under 35 USC 103(a) as being unpatentable over Gierde in view of Sukits as applied to claims 1 and 34 above, and further in view of US Patent Application Publication NO. 2003/0229212 to Fahrner et al. ("Fahrner"). According to the Examiner, Fahrner discloses an ion-exchange chromatography as a competition between an ion and a substrate for a molecule of interest. The Examiner is therefore of the opinion that in light of Gierde and Fahrner, the NaCl competes with the complex to elute a protein in the multi-protein complex, thereby binding to one of the proteins and meeting the claimed limitation. Applicants respectfully disagree for the reasons that follow.

As noted above, Applicants submit that claims 1 and 34 are inventive for the reasons cited above. In particular, Gierde does not teach proper immobilization of *in vivo* formed complexes as described in the present application and the combination of Gierde and Sukits fails to teach the separation of the first ligand from the second ligand while not affecting the bond between the second ligand and the affinity matrix. Further, paragraphs [195-201] of Gierde do not pre-date the present application's priority date and thus are not citable against the present application. Fahrner merely describes ion exchange chromatography in the background and thus does not correct for the deficiencies of Gierde in view of Sukits.

In view of the above, Applicants respectfully submit that claim 37 is inventive over Gierde in view of Sukits and further in view of Fahrner.

The Examiner rejected claims 1-23, 25-26, 34-35 and 38 under 35 USC 103(a) as being obvious having regard to Rigaut et al. (Nature Biotechnology (1999) 17:1030-1032) in view of Sukits and Gierde. The Examiner alleges that it would be obvious to modify Rigaut's method to investigate protein-protein interactions in multi-protein complexes using electrostatic elution. According to the Examiner, the person skilled in the art would modify Rigaut's method to include a NaCl elution step on the multi-protein complex and that the person skilled in the art would be motivated to do so based on Gierde's indication that the nature of multi-protein complexes can be analyzed by eluting individual components. Applicants respectfully disagree for the reasons that follow.

As mentioned above, Applicants respectfully submit that as of the priority date of the present application, Gierde did not disclose that the nature of multi-protein complexes can be analyzed by eluting individual components. Such an indication was only added to Gierde after the present application's priority date. Thus, Gierde is not citable for the purpose of providing this indication against the present application.

Rigaut teaches two purifications via an affinity tag performed one after another (Tandem Affinity Purification). Although during the tandem affinity purification of Rigaut, it is possible that the TAP-tagged target protein would be isolated along with other proteins, there is no indication that the first and second ligands can associate through electrostatic forces or that they can be separated from each other by decreasing the electrostatic force between them. As noted in the previous response dated October 22, 2009, Rigaut analyzes isolated protein complexes by

SDS-PAGE electrophoresis and thus teaches separation of the first ligand from the second by boiling in 1% SDS in the presence of DTT. Such a separation would disrupt any known protein-protein interaction including the bond between the second ligand and the affinity matrix. Thus, the second ligand would not remain immobilized on the affinity matrix as required by present step (d) of claim 1, step (e) of claim 2, step (i) of claim 3 and step (i) of claim 4.

As stated above, the Applicants were the first to demonstrate that elimination of the dynamic range problem by separating the substoichiometrically interacting proteins (i.e. first ligand) from the immobilized fusion protein (i.e. second ligand that remains immobilized) is possible and desirable (see the present application [0004-0015]). Prior to the present application, a person skilled in the art would not expect to be able to separate transient proteins from permanent protein members of such an *in vivo* formed complex.

Even if Gierde paragraphs [195-201] were citable, which we do not agree with, the mere mention of the desire in Gierde at paragraph [0201] provides no guidance that would lead to the presently claimed method. Gierde 0195-0201 and Rigaut do not teach or suggest that the second ligand (the protein that is immobilized on the affinity matrix) must remain immobilized on the affinity column during the elution of the first ligand. As described in the present application, desorption of the second ligand must be avoided, see paragraph [0354] of the present application. Otherwise, a severe dynamic range problem will be encountered, which will make the identification of the first ligand impossible. As stated above, Gierde's statement of "some subset of the complex is released while the rest remains adsorbed" is very vague. Further, as mentioned above, Gierde does not teach or suggest that the elution agent should be chosen in such a way that it favours the separation of the first and second ligand, but does not affect the bond between the second ligand and the affinity matrix.

Thus, none of the cited art disclose or suggest step 1(d) of the presently claimed method.

In addition, Applicants respectfully submit that the main goal of Rigaut was to isolate together the first ligand and the second ligand and thus prove their association *in vivo*. Neither Rigaut nor Gierde teaches or suggests the main motivation and usefulness of claims 1-4, i.e. that only the constituents that are present at substoichiometric amounts must be desorbed and eluted and analysed separately from the high abundance constituents in order to avoid severe high dynamic range problem. See [0005-0015] and [0077- FIG. 21] of the present application.

Thus one of ordinary skill in the art following the teachings of Rigaut would isolate the permanent and transient members of the protein complex, always isolated in different amounts, together and present the results as a strong proof for their interaction/association. However, prior to the present application, only high abundance transient interactors could be detected and the majority of substoichiometrically present transiently interacting proteins were not detected. As mentioned in the previous response dated October 22, 2009, the cause for the failure to detect certain substoichiometrically interacting proteins was not obvious at the priority date of the present application. For example, the weak interactors (i.e. first ligands) could have just been absent after immobilizing the protein of interest (i.e. second ligand) on affinity matrix or, they could have been present at amounts that do not allow their proper analysis and identification. The Applicants were the first to suggest and demonstrate that elimination of the dynamic range problem by separating the substoichiometrically interacting proteins (i.e. first ligand) from the immobilized fusion protein (i.e. second ligand that remains immobilized) is possible and desirable (see patent application [0004-0015] and [0057-0067]).

In view of the above, Applicants respectfully submit that claims 1-23, 25-26, 34-35 and 38 are inventive over Rigaut in view of Sukits and Gierde.

The Examiner rejected claim 24 under 35 USC 103(a) as being unpatentable over Rigaut in view of Sukits and Gierde as applied to claims 1 and 22-23 above, and further in view of US Patent No. 5,007,934 to Stone and US Patent No. 5,849,885 to Nuyens et al ("Nuyens"). As discussed above, paragraphs [195-201] of Gierde do not pre-date the present application's priority date and thus are not citable against the present application. Rigaut in view of Gierde and Sukits further does not teach or suggest step 1(d) separating the first ligand from the immobilized second ligand, which remains bound to the affinity matrix during the separation. The Examiner cites Stone as disclosing using NaCl or KCl as equivalent salts for removing glycoprotein or proteoglycan associated with collagen through electrostatic interaction and cites Nuyens as disclosing NaCl or KCl as equivalent salts for reducing electrostatic interactions between lactoferrin and other proteins. Thus, neither Stone nor Nuyens correct for the deficiencies discussed above of Rigaut in view of Gierde and Sukits.

The Examiner rejected claims 45 and 46 under 35 USC 103(a) as being obvious having regard to Rigaut in view of Sukits and Gierde as applied to claim 1 above, and further in view of Patent 6,610,508 to Hentze et al. and evidenced by US Patent No. 5,753,225 to Clary et al. As

discussed above, paragraphs [195-201] of Gierde do not pre-date the present application's priority date and thus are not citable against the present application. Further, Rigaut in view of Sukits and Gierde do not teach or suggest step 1(d) separating the first ligand from the immobilized second ligand, which remains bound to the affinity matrix during the separation. The Examiner alleges that a person skilled in the art would have found it obvious to modify Rigaut, Sukits and Gierde method to include the step of identifying protein-protein interactions for detecting Alzheimer's disease and that there would be a reasonable expectation of success because protein-protein interaction is a type of ligand-receptor interaction, which is known to be a reversible electrostatic attraction as evidenced by US Patent No. 5,753,225 to Clary et al. The fact that Hentze discloses a step of identifying protein-protein interactions in order to detect disease states including Alzheimer's Disease does not correct for the deficiencies discussed above of Rigaut in view of Sukits and Gierde. The Examiner alleges that "Clary describes receptor-ligand complexes as reversible electrostatic attractions (225 Patent, col 10 ll.66-67; col.11 ll.1-11)". In fact, as noted in the previous response dated October 22, 2009, Clary states that *"Typically, the binding interactions between ligand or peptide and receptor or antigen include reversible noncovalent associations such as electrostatic attraction, Van der Waals forces and hydrogen bonds".* The same statement is true for the permanent protein complexes, as well. Clary does not formulate that reversible protein complexes are held together by predominantly electrostatic forces and that disrupting the electrostatic forces leads to dissociation of the complex. Applicants object that Clary characterizes as "reversible" the physical forces, i.e. electrostatic attraction, Van der Waals forces and hydrogen bonds, however, Clary does not characterize the protein-protein bond as "reversible" or transient. Moreover, Clary does not describe transient protein-protein interactions as predominantly electrostatic and does not suggest the usefulness of a method specifically aimed at detecting transient protein-protein interactions. The Applicants were the first to do that, see [0222] and [0017] in the present application. A skilled artisan would consider that hydrophobic forces, Van der Waals forces and hydrogen bonds are not affected by the salt concentrations that significantly weaken and/or disrupt electrostatic attractions and would expect that disrupting the electrostatic forces is not enough for dissociation of the protein complex. Thus the skilled artisan would have no motivation to try separating the first ligand from the immobilized second ligand.

In view of the above, Applicants respectfully submit that the present claims are inventive over Rigaut in view of Sukits and Gierde alone or having regard further to Hentze, Stone, or Nuyens.

In view of the foregoing, Applicants respectfully request that the rejections under 35 USC 103(a) be withdrawn.

The Commissioner is hereby authorized to charge any fee (including any claim fee) which may be required to our Deposit Account No. 02-2095.

In view of the foregoing comments and amendments, we respectfully submit that the application is in order for allowance and early indication of that effect is respectfully requested. Should the Examiner deem it beneficial to discuss the application in greater detail, he is kindly requested to contact the undersigned by telephone at (416) 957-1678 at his convenience.

Respectfully submitted,

Bereskin & Parr LLP/S.E.N.C.R.L., s.r.l.

By _____

Melanie Szweras
Reg. No. 58,773

Bereskin & Parr LLP/S.E.N.C.R.L., s.r.l.
Box 401, 40 King Street West
Toronto, Ontario
Canada M5H 3Y2

Tel: 416-957-1678
Fax: 416-361-1398